

Dimorphic spermatozoa of the hydrothermal vent prosobranch *Alviniconcha hessleri* : systematic importance and comparison with other caenogastropods

by John HEALY

Abstract. — Ultrastructural features of euspermatozoa and paraspermatozoa of *Alviniconcha hessleri* Okutani & Ohta are described and compared with available data for other gastropods. Euspermatozoa are filiform, 130-135 μm long, with a conical acrosomal vesicle (featuring an apical bleb), a curved, rod-shaped nucleus, an elongate midpiece (with 10-11 helically coiled mitochondrial elements), an annular complex, a glycogen piece and an end piece. An unusual sheath of dense material of uncertain origin is associated with the nucleus and the midpiece. Apart from this sheath, most other features of *Alviniconcha* euspermatozoa occur in euspermatozoa of the Neotaenioglossa (in particular groups such as the Loxonematoidea (Abyssochrysidae), Vermetoidea, Calyptraeioidea, Littorinoidea, Truncatelloidea) and certain Neogastropoda. Paraspermatozoa of *Alviniconcha* consist of large, moderately electron-dense vesicles, smaller, highly electron-dense bodies and occasional mitochondria all enclosed by the plasma membrane. These cells lack axonemes, recognizable nuclear material and an acrosome. Although similar 'nurse cell' type paraspermatozoa associate with euspermatozoa to form spermatozeugmata in the Littorinoidea and Abyssochrysidae, this phenomenon was not observed in *Alviniconcha*. Sperm ultrastructure indicates that *Alviniconcha* belongs within the Neotaenioglossa and is more closely allied to the Littorinoidea-Loxonematoidea than to the Calyptraeioidea or Hipponicoidea. Specifically, sperm data do not support a close relationship between *Alviniconcha* and the Architaenioglossa, the Cerithioidea or the Trichotropidae, but are consistent with a recent proposal to include the genus within the loxonematoidean family Provannidae.

Résumé. — Les caractéristiques ultrastructurales des euspermatozoïdes et paraspermatozoïdes d'*Alviniconcha hessleri* Okutani & Ohta sont décrites et comparées à celles d'autres gastéropodes. Les euspermatozoïdes sont filiformes, et mesurent 130 à 135 μm de long; ils ont une vésicule acrosomiale conique (avec bulle apicale), un noyau en forme de bâtonnet recourbé, une pièce intermédiaire allongée (avec 10-11 éléments mitochondriaux enroulés en hélice), un complexe annulaire, une pièce à glycogène et une pièce terminale. On observe également un manchon inhabituel de matériel dense, d'origine inconnue, associé au noyau et à la pièce intermédiaire. À l'exception de ce manchon, la plupart des caractéristiques des euspermatozoïdes d'*Alviniconcha* se rencontrent également chez les euspermatozoïdes des Neotaenioglossa — en particulier des groupes comme les Loxonematoidea (Abyssochrysidae), Vermetoidea, Calyptraeioidea, Littorinoidea, Truncatelloidea — et des Neogastropoda. Les paraspermatozoïdes d'*Alviniconcha* consistent en un ensemble de grandes vésicules, modérément denses aux électrons, des corps plus petits, très denses aux électrons, et quelques mitochondries, le tout enfermé par la membrane plasmique. Ces cellules n'ont ni axonème, ni matériel nucléaire reconnaissable, ni acrosome. Chez les Littorinoidea et les Abyssochrysidae, de tels paraspermatozoïdes de type « cellules nourricières » s'associent aux euspermatozoïdes pour former des spermatozeugmata : un tel phénomène n'a pas été observé chez *Alviniconcha*. L'ultrastructure des spermatozoïdes montre qu'*Alviniconcha* appartient aux Neotaenioglossa et qu'il est plus proche de la lignée Littorinoidea-Loxonematoidea que des Calyptraeioidea ou des Hipponicoidea. Dans tous les cas, ces données démentent l'idée d'une relation proche entre *Alviniconcha* et les Architaenioglossa, les Cerithioidea ou les Trichotropidae, mais appuient au contraire la proposition récente de classer ce genre dans la famille Provannidae (Loxonematoidea).

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INTRODUCTION

Exploration of deep sea hydrothermal vents continues to reveal an interesting and taxonomically significant array of prosobranch gastropods (FRETTER, 1988; LUTZ *et al.*, 1986; MCLEAN, 1981, 1988*a-c*, 1989; WARÉN & BOUCHET, 1986, 1989). Although the majority of these species are 'archaeogastropods' *sensu lato* (for example see HICKMAN, 1984; MCLEAN & HASZPRUNAR, 1987; MCLEAN 1988*a-b*, 1989) a number of unusual caenogastropods have also emerged from the vent communities (WARÉN & BOUCHET, 1986; WARÉN & PONDER, 1991; BOUCHET & WARÉN, 1991). In describing *Alviniconcha hessleri* from vents in the Mariana Back-Arc Basin, OKUTANI & OHTA (1988) expressed uncertainty as to the true systematic position of their new species and genus. Shell features and the presence of well developed periostracal hairs suggested affinities with the Trichotropidae (Calyptraeioidea), while radular shape and formula indicated a possible connection with the Hipponicoidea (see OKUTANI & OHTA, 1988). Most recently BOUCHET & WARÉN (1991) have placed *Alviniconcha* and *Ifremeria* into the Provannidae, a family established by WARÉN & PONDER (1991) for another hydrothermal vent genus *Provanna*. WARÉN & PONDER (1991) tentatively assigned the Provannidae to the Loxonematoidea to join another family of this relict group, the Abyssochrysidae (see HOUBRICK, 1979). On the basis of comparative anatomy BOUCHET & WARÉN (1991) concluded that any shell similarities between the Provannidae and the Vivipariidae or Trichotropidae were of a superficial nature only. BECK (1991) has, in contrast, suggested that *Olgaconcha* (a synonym of *Ifremeria* Bouchet & Warén, 1991¹) and *Alviniconcha* may be closely related to the Architaenioglossa (eg Vivipariidae) or the Cerithioidea.

Previous studies have shown that sperm morphology in the Mollusca reflects not only the environment of fertilization (eg FRANZÉN, 1955) but also the systematic position of taxa from the level of species upwards (POPHAM, 1979; KOHNERT & STORCH, 1984; HEALY, 1982, 1983*a*, 1986*a, b*, 1988*a, b*, 1990*a-c*; KOIKE, 1985; HODGSON & BERNARD, 1988; HODGSON *et al.*, 1988). In particular, within the prosobranch order Caenogastropoda (= Mesogastropoda + Neogastropoda), a reasonably good data base now exists with which to test views on the likely position of newly discovered taxa such as *Alviniconcha*.

During the course of the present study it became obvious that sperm ultrastructural data for the Trichotropidae would be needed for comparison with *Alviniconcha*. I have therefore included herein a brief description and salient micrographs of the euspermatozoa and paraspermatozoa of a representative trichotropid, *Trichosirius inornatus* (Suter) from New Zealand. It is hoped that this study may stimulate further ultrastructural work on sperm morphology of hydrothermal vent molluscs.

MATERIALS AND METHODS

Live *Alviniconcha hessleri* Okutani & Ohta, 1988 were collected during the BIOLAU cruise of the Lau Basin, south-east of Fiji, using the submersible craft 'Nautilé' (12-27 May,

1. Dr. P. BOUCHET advises (in litt.) that *Olgaconcha tufari* Beck, 1991 is a synonym of *Ifremeria nautiléi* Bouchet & Warén, 1991. The paper by BOUCHET & WARÉN (1991) was published before that of BECK (1991), hence the name *Ifremeria nautiléi* Bouchet & Warén must take priority over *Olgaconcha tufari* Beck.

1989). Collection data for this material are as follows : dive 12 on site Vailili, Valufa Ridge, Lau Basin, 23°13' S, 176°38' W at a depth of 1,750 m (Observer for this dive was P. CRASSOUS, IFREMER, Brest). The material examined herein consists of sperm-filled, sac-like outpockets of the distal end of the sperm duct (as described by WARÉN & BOUCHET, 1992). Voucher specimens of *Alviniconcha hessleri* have been deposited in the Muséum national d'Histoire naturelle (Paris). Tissues were fixed in cold (0-4°C) cacodylate-buffered glutaraldehyde then dispatched to the author for subsequent processing and analysis. Small pieces of the sperm sacs were taken from the samples provided and processed for transmission electron microscopy (TEM) according to the following schedule : (1) rinse in cold 0.1 M cacodylate buffer containing 10 % sucrose (1 hr); (2) osmication of tissues for two hours (1 % osmium tetroxide in cacodylate or phosphate buffer); (3) buffer rinse; (4) gradual dehydration using ethanol; (5) embedding in Spurr's epoxy resin. For comparison with *Alviniconcha hessleri*, portions of the sperm duct of a representative trichotropid, *Trichosirius inornatus* (Suter), were processed for TEM. This material derives from sea water formalin-fixed specimens collected by W. F. PONDER (June 1966). After rinsing in phosphate buffer, tissue pieces of *Trichosirius inornatus* were processed as outlined above for *Alviniconcha hessleri*. All ultrathin and semithin sections were prepared with an LKB IV Ultratome. Ultrathin sections were stained using DADDOW's (1986) modified double lead procedure and examined with a Hitachi 300 TEM operated at 75kV. Tissue squashes were also examined by standard phase-contrast as well as Nomarski-phase contrast light microscopy using an Olympus BH2 light microscope.

RESULTS

EUSPERMATOZOA

Euspermatozoa of *Alviniconcha hessleri* (hereafter referred to as *Alviniconcha*) are filiform, approximately 130-135 μm long and comprise an acrosomal complex, nucleus, midpiece, annular complex, glycogen piece and end piece.

Acrosomal complex : The acrosomal vesicle is conical, 0.9 μm long and posteriorly circular in transverse profile (pl. I C-F). It rests on a thin, basal plate which is grooved to fit the nuclear apex (pl. I E, F). An accessory membrane attached to the basal plate sheaths the posterior 0.5 μm of the acrosomal vesicle (pl. I D-F). Anteriorly the vesicle membrane expands away from the vesicle contents to form an electron lucent space : the apical bleb (pl. I B, E, F). Oblique transverse sections reveal the presence of radially arranged plates within the matrix of the acrosomal vesicle (pl. I D). A basal invagination, extending almost to the apex of the vesicle, contains subacrosomal material organized as an axial rod embedded in a granular matrix (pl. I C-F).

Nucleus : TEM confirms light microscopic observations that the euspermatozoan nucleus of *Alviniconcha* is slightly curved (pl. I A). The nucleus is cylindrical (diameter 0.4 μm posteriorly, tapering to 0.2 μm anteriorly) and approximately 7.5 μm long, with narrow basal invagination (0.76 μm deep) which forms the attachment site for the centriolar derivative and axoneme (pl. I A, G, H; II A, E). An extensive, irregularly shaped sheath of finely granular

material is associated with the surface of the nucleus, particularly in the middle to basal regions where nuclear contents sometimes appear fibrous in substructure (pl. I G-J; II A). Although 'ballooning' of the plasma membrane away from the surface of the nucleus (pl. I A, I, pl. II A) could prove to be an artifact of fixation, the localization of this phenomenon, and the generally good preservation of other cell components tend to argue against this. As plate I and plate II show, the dense sheath penetrates into the anterior region of the midpiece where it partially surrounds the axoneme, and frequently the mitochondrial elements themselves. The absence of developing euspermatozoa prevents any resolution of the origin or purpose of the sheath substance. The varied shape and thickness of the sheath suggests either that this structure is genuinely variable in its morphology or that it may not have reached its final form (i.e. the observed euspermatozoa are still in the process of maturation).

Midpiece : The midpiece exhibits ten to eleven mitochondrial elements which wind helically around a 9 + 2 pattern axoneme (pl. I G, H, K; II C-F). Each element has flattened adjoining surfaces and contains scattered, poorly developed cristae (pl. II C-F). As previously mentioned the dense sheath observed in the nuclear region also occurs within the midpiece and the nucleus-midpiece junction (pl. I G, H, K; II E, D). Longitudinal sections through the anterior portion of the midpiece show that the sheath surrounding the midpiece elements or the axoneme is often discontinuous suggesting segmentation (pl. I G, H; II D). In some euspermatozoa, sheath material associated with the axoneme appeared to be helically arranged (see pl. II F). The midpiece measures between 76 and 80 μm in length (light microscopic observations).

Annular complex, glycogen piece, end piece : The annular complex occurs at the junction of midpiece and glycogen piece. It comprises a dense ring or 'annulus' (applied to the inner surface of the plasma membrane) and a closely associated collar of dense material which lines the terminal 0.4 μm of the mitochondrial membrane (pl. III A). Within the glycogen piece each of the nine axonemal doublets is associated with a tract of dense, putative glycogen granules (pl. III A, B). As plate III C shows, the glycogen piece ends abruptly, leaving the end piece of the euspermatozoon to consist solely of the plasma membrane and axoneme. The combined length of the glycogen piece and end piece varies from 46 to 50 μm (light microscopic observations).

PARASPERMATOZOA

Paraspermatozoa of *Alviniconcha hessleri* are rounded cells containing the following features : (1) a variable number (5-8) of large, membrane-bound, spherical vesicles; (2) one to three spherical, highly electron dense bodies, (3) a finely granular, background matrix (4) a delimiting, plasma membrane (pl. III D, E). Mitochondria were only rarely observed. No evidence of an acrosome, axonemes (either contained within the parasperm body or emergent as tails) or centrioles could be found. In spite of the absence of developing paraspermatozoa in material studied, comparison with other caenogastropods suggests that the larger vesicles are probably products of Golgi and/or endoplasmic reticular activity. The origin of the smaller, more highly electron-dense bodies (pl. III D, E) is more difficult to determine. There remains the possibility that these bodies are condensed nuclear remnants (caryomerites' of authors) but

the absence of a double nuclear membrane around the bodies or any evidence of fibrillar/coarse granular substructure or lacunae argue against this view. Wherever the large vesicles touch, their surfaces are flattened (pl. III D). Undoubtedly this affords efficient packing of vesicles and a certain degree of structural integrity for the paraspermatozoon as a whole. Paraspermatozoa and euspermatozoa of *Alviniconcha hessleri* were never observed in contact with each other, suggesting that spermatozeugmata are probably not formed in this species.

TRICHOTROPIDAE
(*Trichosirius inornata* Suter)

EUSPERMATOZOA

Acrosomal complex : The acrosomal vesicle is conical and deeply invaginated with a prominent apical bleb (pl. IV A). It has a maximum diameter of $0.3\ \mu\text{m}$ near the base, and average length of $1.0\ \mu\text{m}$. Apically the vesicle is sometimes curved or slightly hooked, suggesting that this organelle is flexible. A rod-shaped deposit of subacrosomal material runs almost the full length of the vesicle invagination ($0.67\text{-}0.7\ \mu\text{m}$). A thin, centrally perforate, basal plate lies between the basal rim of the acrosomal vesicle and the nuclear apex. Although longitudinal sections suggest the presence of an accessory membrane, the precise extent of this feature could not be determined.

Nucleus : The euspermatozoon nucleus is elongate (approximately $50\text{-}54\ \mu\text{m}$ long, light microscopic observations), tubular and encloses the centriolar derivative and anterior portion of the axoneme (pl. IV A-C, F). Nuclear contents are highly electron dense and decrease in extent anteriorly until only a thin layer ($0.02\ \mu\text{m}$ thick) surrounds the centriolar derivative (pl. IV A, C, F). Nine microtubular elements embedded in dense material constitute the centriolar derivative, however the exact substructure of these elements was not determined. The axoneme appears to show the typical $9 + 2$ configuration. Diameter of the nucleus, including the nuclear and plasma membranes and enclosed axoneme decreases from $0.56\ \mu\text{m}$ posteriorly to $0.33\ \mu\text{m}$ near the acrosome.

Midpiece : The midpiece begins immediately posterior to the nucleus and comprises the axoneme (continuing from the nuclear region) surrounded by five to six helically coiled, mitochondrial elements (pl. IV B, D, E, G). Each of the mitochondrial elements exhibits random, unmodified cristae (pl. IV D, E). The midpiece measures approximately $12\ \mu\text{m}$ (light microscopic observations).

Annular complex, glycogen piece : Longitudinal or oblique longitudinal sections revealed that an annular complex is present at the junction of the midpiece and glycogen piece. This complex consists of two distinct rings, each connected to the inner surface of the plasma membrane and each showing a triangular profile in longitudinal section (pl. IV E). The glycogen piece is recognized by the nine spoke-like structures connecting the doublets with the plasma membrane (pl. IV G). Although transverse sections were obtained showing evidence of periaxonemal granules attached to the spokes most euspermatozoa seemed to lack such

deposits. This is almost certainly due to the fixation deficiencies of the formalin preserved material available for study. The numerous individual axonemes observed (all associated with disrupted plasma membranes) are here interpreted as the terminal or end piece of the euspermatozoon. Light microscopy gives a length of $12\mu\text{m}$ for the glycogen piece and end piece combined.

PARASPERMATOZOA

Paraspermatozoa of *Trichosirius inornatus* are vermiform cells, 24 to $32\mu\text{m}$ long (light microscopic observations) which are composed of longitudinally aligned axonemes, scattered mitochondria, numerous oblong, dense vesicles and a granular cytoplasm (pl. IV F, G). Transverse sections show that mature cells are laterally compressed, but moreso anteriorly where mitochondria are positioned between the axonemes (pl. IV F). All parasperm organelles are contained within a common plasma membrane. Axonemes are positioned peripherally, close to or in contact with the inside surface of the plasma membrane. Towards the posterior region of the cell, the axonemes project into shallow outpockets of the plasma membrane (pl. IV F, G). No evidence of physical contact between paraspermatozoa and euspermatozoa (eg spermatozeugmata formation) was observed in *Trichosirius inornatus*.

DISCUSSION

STRUCTURAL COMPARISON

The presence of paraspermatozoa and more importantly the morphology of the euspermatozoa clearly identifies *Alviniconcha* as a caenogastropod. Caenogastropods can be divided into two main groups on the basis of euspermatozoan features, notably the substructure of the acrosome and midpiece (HEALY, 1988a). These groups are : (1) the Cerithioidea, Ampullarioidea and Cyclophoroidea and (2) the remainder of the Caenogastropoda. The first of these groups is characterized by absence of an apical bleb and accessory membrane in the acrosomal complex and presence of highly organized, parallel cristal plates in the mitochondrial elements. *Alviniconcha* belongs to the second, larger group of caenogastropods, the euspermatozoa of which possess an apical bleb (sometimes poorly developed), an accessory membrane (also present in the enigmatic bridging group Campaniloidea — see HEALY, 1986b), and 5 to 11 helically coiled mitochondrial elements (cristae usually unmodified, sometimes tubular). In all caenogastropod euspermatozoa with the exception of the truncatelloidean *Stenothyra* sp. (HEALY, 1983b), the glycogen piece exhibits nine periaxonemal tracts of granules. Elsewhere within the Mollusca this arrangement of sperm glycogen deposits occurs in the Octopoda (LONGO & ANDERSON, 1970).

Euspermatozoa of *Alviniconcha* differ from those of other caenogastropods in possessing a well developed sheath of electron dense material which extends from the nucleus into the anterior portion of the midpiece. An absence of spermiogenic stages in material available for

study prevented any determination of the origin of this dense sheath. The frequently observed 'disjointed' nature of the sheath within the *Alviniconcha* midpiece invites comparison with the segmented midpiece sheath observed in euspermatozoa of the relict campaniloidean *Campanile symbolicum* Iredale (see HEALY, 1986b). Although the origin of the *Campanile* sheath is also unknown, it is, in contrast to the *Alviniconcha* sheath, composed of equal-sized elements which are membrane bound and never associated with the nucleus. In *Alviniconcha* the dense sheath is well developed in the region of the nucleus (actually contacting the nuclear surface), while posteriorly, it often extends around the axoneme and midpiece elements. Spermatozoa of certain stylommatophoran pulmonates also possess a non-membrane bound perinuclear sheath which may be layered (eg *Helix aspersa* (Müller); HEALY & JAMIESON, 1989) or exhibit paracrystalline substructure (eg *Oxyloma elegans* (Risso); SELMI *et al.*, 1989). In *Oxyloma elegans*, the sheath disappears during storage in the seminal receptacle (SELMI *et al.*, 1989). Unlike *Alviniconcha*, the sheath in pulmonate sperm does not also extend into the midpiece. The marked variation in thickness and distribution of the dense sheath in *Alviniconcha* suggests that this structure perhaps has not attained its final form (? a late spermiogenic feature) or alternatively, is genuinely variable in its morphology. Detailed study of spermatids and seminal receptacle spermatozoa will help determine the origin and possible function of the sheath material. Even if the observed sheath is not fully mature in its form or proves to be a transient feature, its presence distinguishes *Alviniconcha* from other caenogastropods. I will return to the possible taxonomic implications of *Alviniconcha* eusperm morphology in the final section of this discussion.

Paraspermatozoa of *Alviniconcha* closely resemble those of the Littorinoidea (especially *Littorina*, see BUCKLAND-NICKS & CHIA, 1977) and the loxonematoidean *Abyssochrysos brasilianum* Bouchet (see HEALY, 1990a). In paraspermatozoa of all three taxa, an acrosome, axonemes and centrioles are absent, and large secretory vesicles suspended within a granular matrix constitute the bulk of the spherical cell body. In *Abyssochrysos*, the littorinid subfamily Littorininae and the atlantoidean *Carinaria lamarcki* Péron & Lesueur, euspermatozoa attach in an organized cluster to each paraspermatozoon to form spermatozeugmata (REINKE, 1912; BUCKLAND-NICKS & CHIA, 1977; THIRIOT-QUIEVREUX & MARTOJA, 1979; KOHNERT & STORCH, 1984; REID, 1986; HEALY, 1990a). Spermatozeugmata were not observed in *Alviniconcha* but this is also the case in certain littorinids which possess 'nurse cell' type paraspermatozoa such as the genus *Peasiella* (REID, 1989) and probably the genus *Provanna* (Provannidae, Loxonematoidea — see WARÉN & PONDER, 1991). Although the large vesicles of *Alviniconcha* paraspermatozoa are probably products of Golgi and/or endoplasmic reticular activity (as occurs in other caenogastropods), the likely origin of the highly electron-dense bodies is problematical. Two alternatives seem possible. Either these bodies represent condensed nuclear remnants (= 'caryomerites') or they are another form of secretory product. Observations on parasperm development would help to clarify this issue, but for the present it is worth noting that the dense bodies are enclosed by a single and not a double (eg nuclear) membrane and that the bodies show no evidence of nuclear substructure such as fibrils, coarse granules or lacunae. Although paraspermatozoa of most littorinids and *Abyssochrysos* do not retain a nuclear fragment (BUCKLAND-NICKS & CHIA, 1977; REID, 1986; HEALY, 1990a) some species do (REID, 1986). Retention of condensed nuclear material in caenogastropod paraspermatozoa is not unusual for certain taxa (eg Cyclophoroidea, Cerithioidea; SELMI & GIUSTI, 1980; HEALY, 1986a, 1988a).

SYSTEMATIC IMPLICATIONS

The systematic position of *Alviniconcha* was briefly discussed by OKUTANI & OHTA (1988) in their account of the new species and genus. They suggested a possible link between *Alviniconcha* and the calyptraeoidean family Trichotropidae (conchological features including prominent 'hairy' periostracum) or, in view of radular features perhaps a closer connection between the genus and the Hipponicoidea¹. BECK (1991) believes that *Alviniconcha* and *Olgaconcha* (a synonym of *Ifremeria* Bouchet & Warén, 1991; see footnot 1, p.274) may be closely related to the Architaenioglossa (Vivipariidae and allies) or the Cerithioidea. A stronger case, favouring loxonematoidean affinities for *Alviniconcha* has been put forward by WARÉN & BOUCHET (1992).

Comparative sperm data for the Caenogastropoda, despite being fragmentary for the Calyptraeoidea and Hipponicoidea, provide some basis for evaluating these differing views concerning the relationships and systematic position of *Alviniconcha*. To begin with, let us consider the possibility of a Calyptraeoidea or Hipponicoidea connection. Some genera of the Calyptraeoidea (for example *Calyptraea*, *Crepidula*) possess a short eusperm nucleus (KOHNER & STORCH, 1984) like *Alviniconcha*, while in other genera such as *Trichosirius* (this study) and the Hipponicoidea (*Vanikoro*; HEALY, 1984) it is long and tubular. The value of eusperm nuclear shape in the Caenogastropoda is limited, for in some families (eg Littorinidae, Turridae, Eulimidae, Terebridae) different genera may show short or tubular nuclei (although the shape appears consistent in any given genus) (HEALY, 1984, 1988a; KOHNER & STORCH, 1984; KOIKE, 1985). Other euspermatozoan features such as the acrosomal complex, midpiece and glycogen piece of *Alviniconcha* are similar to those seen in the Calyptraeoidea, Hipponicoidea and in fact many other caenogastropod superfamilies (BULNHEIM, 1962; HEALY, 1984, 1988a, b, 1990b; KOHNER & STORCH, 1984; KOIKE, 1985). The dense sheath of *Alviniconcha* euspermatozoa is not, however, seen in any other group (the segmented sheath of *Campanile* being here regarded as non-homologous). More significantly perhaps than eusperm morphology, the paraspermatozoa of the Calyptraeoidea (*Crepidula*, GOULD, 1917; *Trichosirius*, this study) are vermiform cells with multiple, internal axonemes and small secretory vesicles. This type of paraspermatozoon, also recorded in other caenogastropod groups such as the Cypraeoidea, Doliioidea, Muricoidea (MELONE *et al.*, 1980; HEALY, 1988a), contrasts with the round paraspermatozoa of *Alviniconcha* which exhibit large secretory vesicles lack axonemes. No data on the presence or structure of paraspermatozoa in the Hipponicoidea or the Fossaridae are at present available, the latter family being transferred to the Cerithioidea (now as subfamily of Planaxidae; see footnote 1, p. 274). To sum up, eusperm and parasperm structure do not provide any overwhelming support for a connection between *Alviniconcha* and the Calyptraeoidea or the Hipponicoidea (specifically the Trichotropidae).

A large body of information now exists on the fine structure of euspermatozoa and paraspermatozoa in the Architaenioglossa (Ampullarioidea, Cyclophoroidea) and the Ceri-

1. In their discussion of the relationships of *Alviniconcha*, OKUTANI & OHTA (1988) cite the Fossaridae as a member family of the Hipponicoidea. Anatomical studies have shown that species of the genus *Fossarus* share a cephalic brood pouch with the Planaxidae, and partly on this basis the Fossaridae were transferred to the Cerithioidea (PONDER, 1980; PONDER & WARÉN, 1988). HOUBRICK (1990) has subsequently reduced the Fossaridae to the status of subfamily within the Planaxidae.

thioidea (for principal references see HEALY, 1983a, 1986a, 1988a; KOHNERT & STORCH, 1984; KOIKE, 1985). These groups are united by many similarities in eusperm and parasperm morphology, but do not share any significant similarities with either the euspermatozoa or paraspermatozoa of *Alviniconcha*. On this basis I cannot support BECK's (1991) suggestion that *Alviniconcha* or *Olgaconcha* (= *Ifremeria*) may be closely related to the Architaenioglossa or the Cerithioidea.

A further view, that of an *Alviniconcha*-Loxonematoidea relationship (proposed by BOUCHET & WARÉN, 1991) seems much more promising than those already considered above, primarily because the only caenogastropod known to possess similar euspermatozoa and paraspermatozoa to *Alviniconcha* is *Abyssochrysos brasilianum* Bouchet (Abyssochrysidae, Loxonematoidea). There are a few differences however. Unlike *Alviniconcha*, euspermatozoa and paraspermatozoa of *Abyssochrysos* definitely form spermatozeugmata similar to those of many Littorinidae (HEALY, 1990a; see also BUCKLAND-NICKS & CHIA, 1977). Secondly, the dense sheath of *Alviniconcha* euspermatozoa is lacking in *Abyssochrysos*. Absence of spermatozeugmata in *Alviniconcha* may not be as taxonomically significant as it first appears because the same situation has been noted in *Provanna* (recently transferred to Loxonematoidea) and in certain littorinids (REID, 1989; WARÉN & PONDER, 1991). Eusperm nuclei of *Provanna* differ from those of *Alviniconcha* in being elongate and tubular rather than short and solid (HEALY, 1990c).

In conclusion, sperm morphology supports a close relationship between deep-sea taxa such as *Alviniconcha*, *Abyssochrysos* and *Provanna*, and other basal neotaenioglossans notably the Littorinoidea. BOUCHET & WARÉN (1991) have grouped *Provanna*, *Alviniconcha* and *Ifremeria* within the Provannidae (of WARÉN & PONDER, 1991), and following WARÉN & PONDER, tentatively included this family in the Loxonematoidea (to join the Abyssochrysidae). In spite of anatomical differences, the Littorinoidea and Loxonematoidea (now including Abyssochrysidae and Provannidae) significantly exhibit the same eusperm and parasperm morphology. This similarity may prove to be due to symplesiomorphy, although it is by no means certain that the 'nurse cell' paraspermatozoon of the Littorinoidea and Loxonematoidea is in fact a basal, unspecialized type relative to the axonemal paraspermatozoa of other caenogastropods (see JAMIESON, 1987 for further discussion). In other lower neotaenioglossan superfamilies, paraspermatozoa appear to be absent in the Truncatelloidea and Cingulopsoidea (HEALY, 1984; but see PONDER, 1983 for the possibility of parasperm in Barleceidae) while euspermatozoa and paraspermatozoa of the Cerithioidea and Architaenioglossa show numerous differences from those of the Littorinoidea and Loxonematoidea (see HEALY, 1983a, 1986a, 1988a for comparison and further references). Undoubtedly as knowledge of sperm structure in basal groups within the Neotaenioglossa improves, a clearer picture of the phylogeny of this caenogastropod assemblage will emerge.

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PLATE I

Alviniconcha : euspermatozoa

- A — Longitudinal section (L.S.) through acrosome, nucleus and proximal portion of midpiece. Note expanded plasma and nuclear membranes (*).
- B-C — Series of transverse sections (T.S.) from apex to base of acrosomal complex. Plate substructure visible in wall of vesicle in pl. I D (arrow heads).
- D, E — L.S. acrosomal complex and nuclear apex of two euspermatozoa.
- G, H — L.S. junction of nuclear invagination and proximal portion of midpiece. Note disjointed nature of dense sheath associated with axoneme and mitochondrial elements.
- I, J — T.S. nuclei and dense sheath.
- K — T.S. junction of nucleus and midpiece.

Scale bars : A = 1.0 μ m; G, H = 0.5 μ m; B-F, K, J = 0.25 μ m

Abbreviations used in the figures : a, acrosomal complex; ab, apical bleb; am, accessory membrane (of acrosomal complex); an, annular complex; ar, axial rod (of acrosomal complex); av, acrosomal vesicle; ax, axoneme; bp, basal plate (of acrosomal complex); c, centriolar derivative; ds, dense sheath (of euspermatozoon); ep, end piece; eu, euspermatozoa; gp, glycogen piece; m, unmodified mitochondrion; M, midpiece; mpe, midpiece (mitochondrial) elements; n, nucleus; p, paraspermatozoon; pm, plasma membrane; v, oblong vesicles.

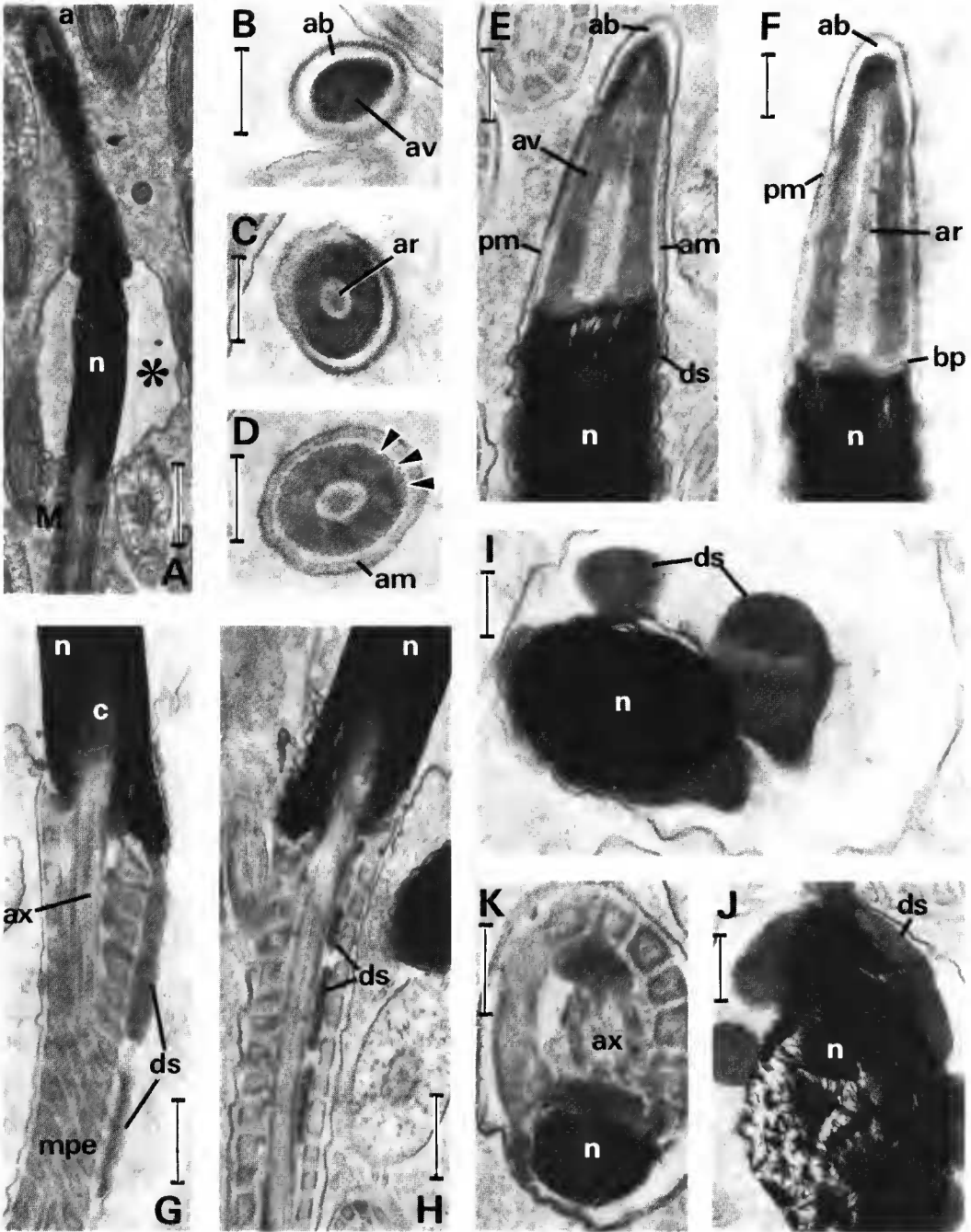


PLATE I

PLATE II

Alviniconcha : euspermatozoa

- A — L.S. base of nucleus showing expanded nature of plasma and nuclear membranes (*).
 - B — T.S. nucleus and dense sheath.
 - C — T.S. midpiece. Note dense sheath associated with periaxonemal surface of mitochondrial elements.
 - D — L.S. proximal portion of midpiece showing dense sheath associated with mitochondrial elements and axoneme.
Inset : T.S. same region of midpiece.
 - E — L.S. junction of nucleus and midpiece showing seemingly 'fluid' nature of dense sheath.
 - F — L.S. midpiece showing slight spiralling of dense sheath around axoneme. Inset : same region of midpiece.
- Scale bars : A = 0.5 μ m; B-F = 0.25 μ m.

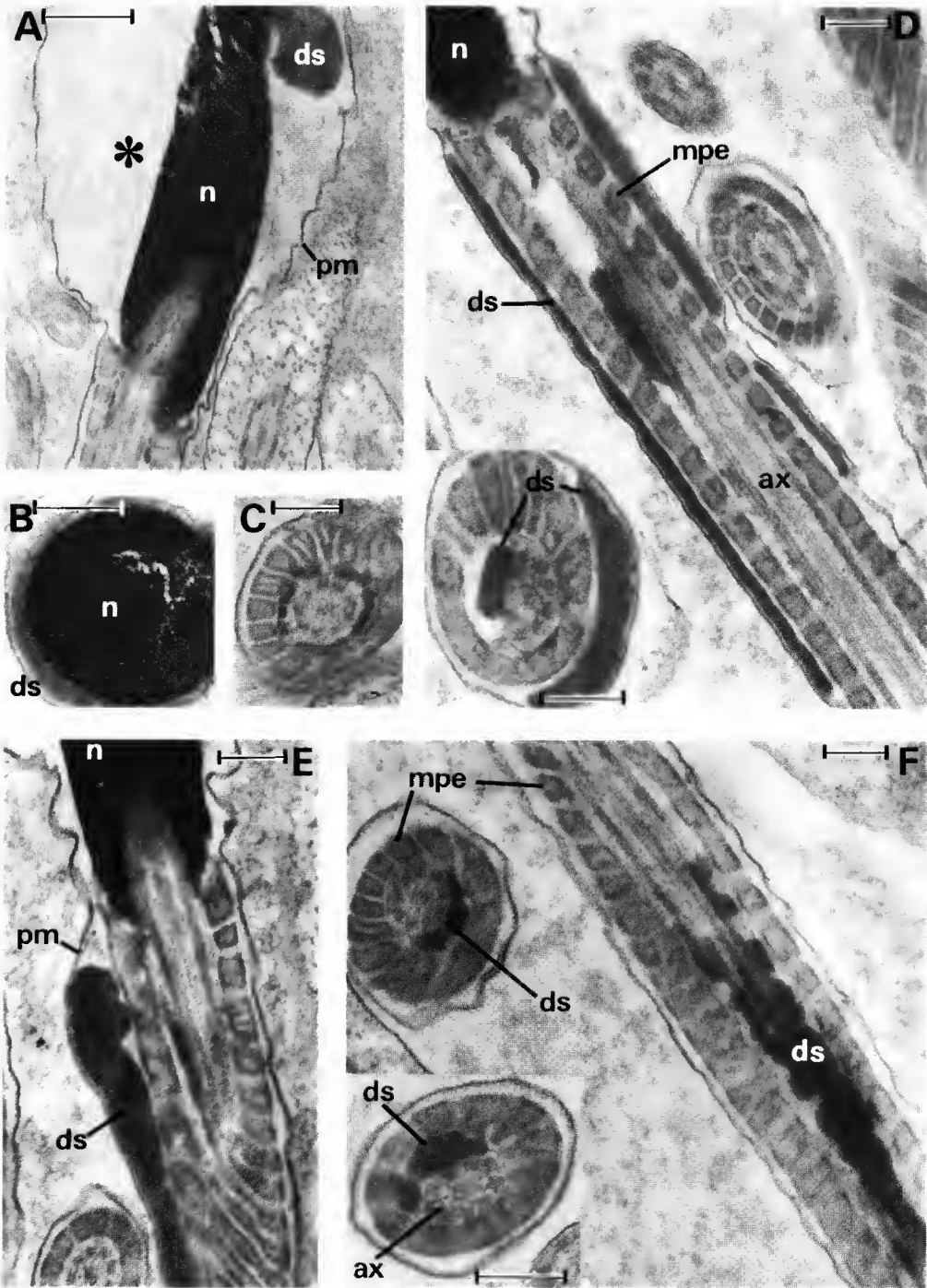


PLATE II

PLATE III

Alviniconcha : cuspermatozoa and paraspermatozoa

- A — L.S. junction of midpiece and glycogen piece showing annular complex.
- B — T.S. junction of nucleus and midpiece (upper right), midpiece and glycogen piece.
- C — L.S. junction of glycogen piece and end piece.
- D — Paraspermatozoa. Note large vesicles pressed against each other, and occasional, highly electron-dense bodies (arrow). Inset showing three dense bodies within a common membrane.
- E — Detail of dense body and portion of a large vesicle.

Scale bars : A-C = $0.5\mu\text{m}$; D-E = $1.0\mu\text{m}$.

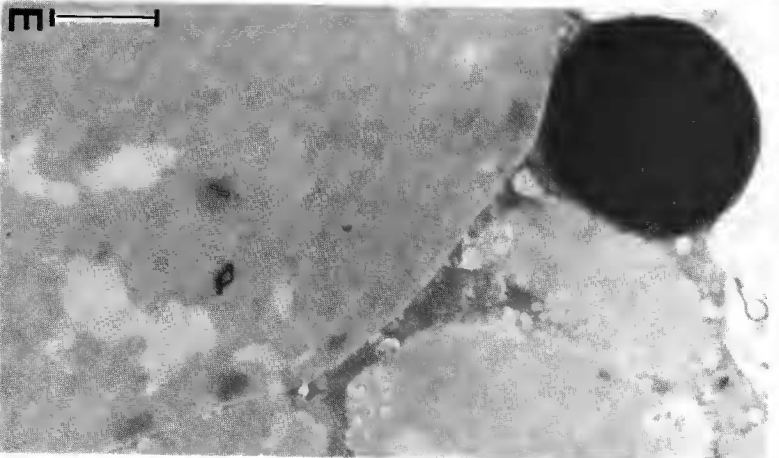
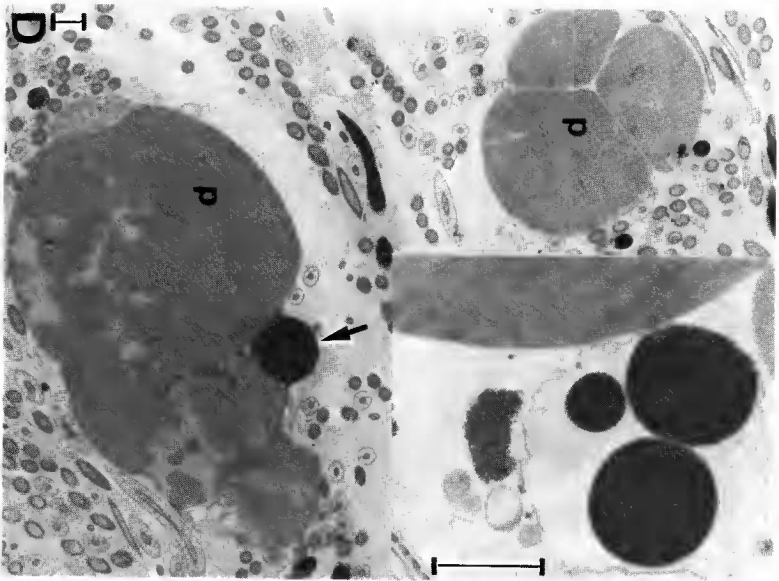
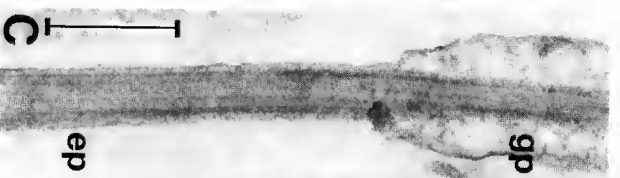
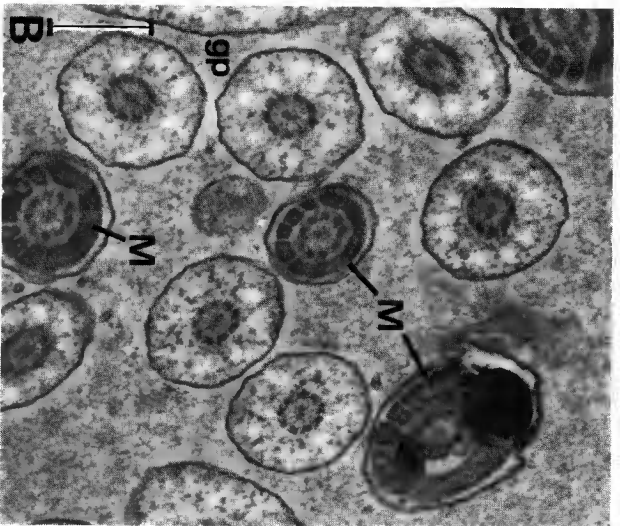
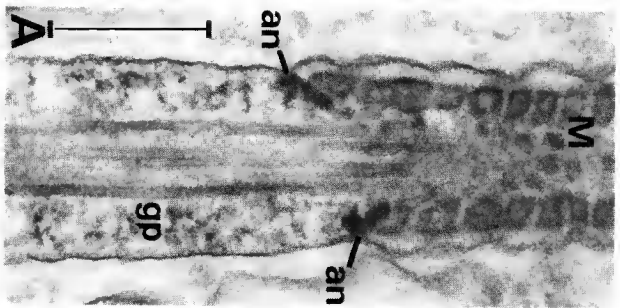


PLATE III

PLATE IV

Trichosirius inornatus : euspermatozoa and paraspermatozoa

- A — L.S. through eusperm acrosomal complex and nuclear apex.
 - B — L.S. through eusperm nucleus-midpiece junction.
 - C — T.S. through eusperm nuclear apex and centriolar derivative.
 - D — T.S. eusperm midpiece showing 5-6 helical, mitochondrial elements.
 - E — L.S. junction of midpiece and glycogen piece showing annulus (2 rings) and helical mitochondrial elements.
 - F — T.S. eusperm nucleus (in region of centriolar derivative) and flattened anterior region of paraspermatozoon (note interspersed axonemes and mitochondria).
 - G — T.S. various regions of euspermatozoa and paraspermatozoa. 1-3 indicate anterior-posterior sequence through paraspermatozoa. Note spoke-like structures within the glycogen piece of euspermatozoa.
- Scale bars : A-E = 0.25 μ m; F = 0.5 μ m.

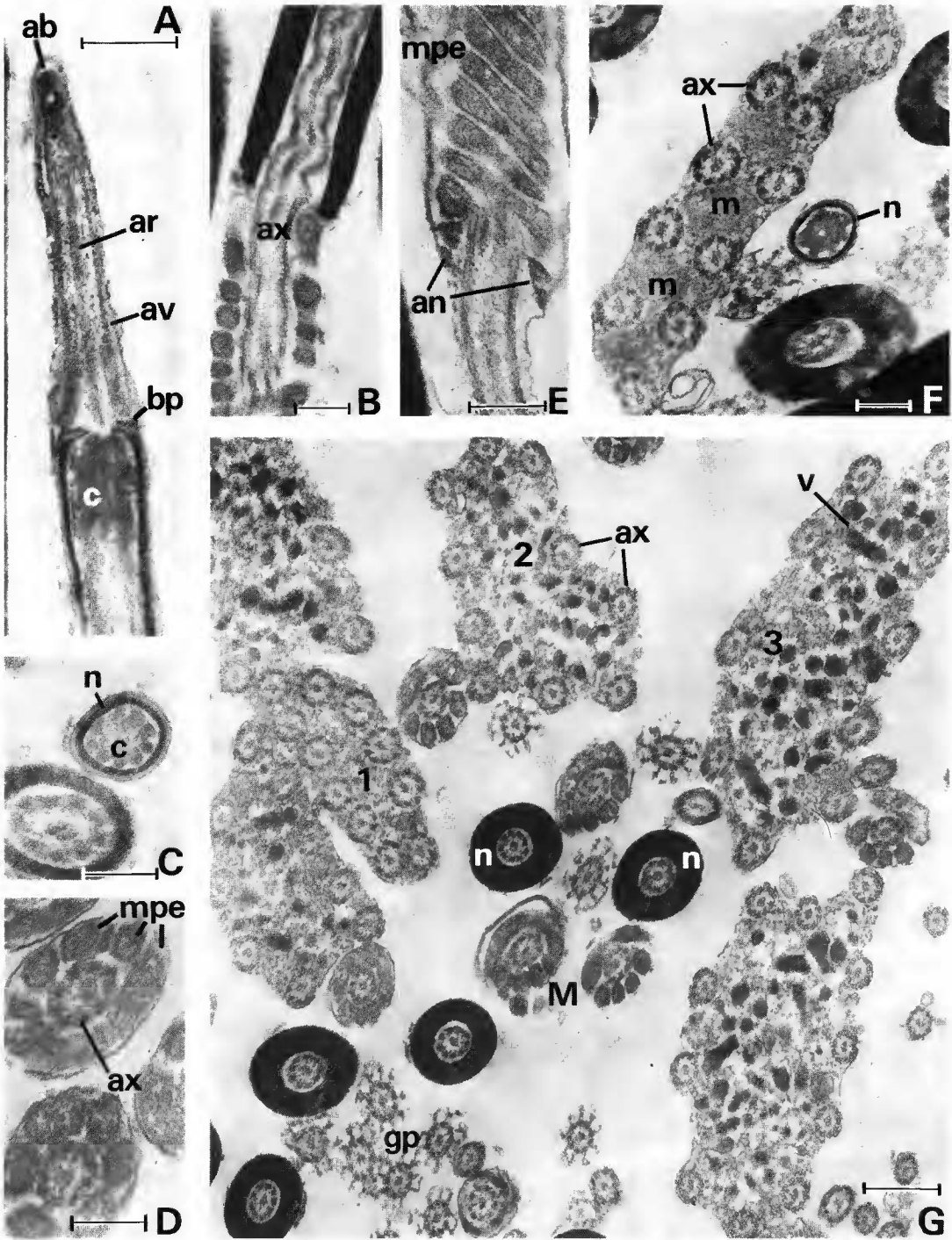


PLATE IV